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REVIEW ARTICLE

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Oncolytic adenovirus: A tool for cancer therapy in combination with other therapeutic approaches

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Abstract

Cancer therapy using oncolytic viruses is an emerging area, in which viruses are engineered to selectively propagate in tumor tissues without affecting healthy cells. Because of the advantages that adenoviruses (Ads) have over other viruses, they are more considered. To achieve tumor selectivity, two main modifications on Ads genome have been applied: small deletions and insertion of tissue- or tumor-specific promoters. Despite oncolytic adenoviruses ability in tumor cell lysis and immune responses stimulation, to further increase their antitumor effects, genomic modifications have been carried out including insertion of checkpoint inhibitors and antigenic or immunostimulatory molecules into the adenovirus genome and combination with dendritic cells and chemotherapeutic agents. This study reviews oncolytic adenoviruses structures, their antitumor efficacy in combination with other therapeutic strategies, and finally challenges around this treatment approach.

KEYWORDS

adenovirus, dendritic cells, immune responses, oncolytic viruses, tumor

1 | INTRODUCTION

Despite attempts and advances in treatment, cancer remains one of the leaders of mortality and morbidity worldwide and has many adverse socioeconomic effects (Viegas, Ladeira, Costa-Veiga, Perelman, & Gajski, 2017). In addition to chemotherapy and radiotherapy that are considered as conventional cancer treatments, other approaches have been emerged to combat cancer such as immunotherapy and virotherapy (Simpson, Relph, Harrington, Melcher, & Pandha, 2016). Although Oncorine (H101), an oncolytic adenovirus, was approved by Chinese state FDA for head and neck carcinomas in 2005, the fist approved oncolytic virus by FDA was a genetically engineered herpes simplex virus named talimogene laherparepvec, which was approved in 2015 for melanoma patients (Gopisankar & Surendiran, 2018). Among different viruses, using adenoviruses (Ads) have attracted much attention because of their ability to grow in high titers in vitro, to replicate in episomal form, and to upregulate costimulatory molecules and induce chemokine and cytokine responses, which introduce them as "nature's adjuvants" (Robert-Guroff, 2007). The Ads were first isolated in 1953 from human adenoid cells (Rowe, Huebner, Gilmore, Parrott, & Ward, 1953) and thus far, more than 100 species have been identified, and of them, human Ads are classified into seven subgroups (A–G). Among them, serotype 5 (Ad5) which belongs to subgroup C is more extensively used in gene therapy and virotherapy (Rux & Burnett, 2004). This review will focus on Ad genome structure, oncolytic adenovirus construction, and their therapeutic efficacy in combination with other anticancer agents.

2 | ADENOVIRUS

The Ads are non-enveloped viruses with a double-stranded linear DNA (about 36 kb) enclosed by an icosahedral protein capsid. The viral genome consists of the early genes (*E1*, *E2*, *E3*, and *E7*) and late genes (*L1*, *L2*, *L3*, *L4*, and *L5*) (Russell, 2000). To infect host cells, fiber knob domain of Ads binds to coxsackie-adenovirus receptor on the host cells, then the interaction between arginine-glycine-aspartic acid (RGD) motif on the penton base of the virus and cellular integrins helps internalization of the virus (Alemany, 2014). Following the infection of the target cell and disassembly of virus in early endosome, viral DNA enters nucleus through pores with the aid of microtubules and dynein and triggers transcription of early gene *E1A*, a subunit of *E1* gene, which controls the cell cycle and expression of the other early genes (Alemany, 2009). In addition, because of binding to retinoblastoma (Rb), *E1A* releases *E2F* from Rb, which is necessary for the synthesis of S phase components (Russell, 2000). On the other hand, induction the expression of p14ARF by *E1A* leads to accumulation of p53 in the cell nucleus and therefore growth arrest (de Stanchina et al., 1998). To overcome this problem, Ads encode another early gene called the *E1B* gene, which has two major polypeptides: E1B19K and E1B55K (Cheng, Wechman, McMasters, & Zhou, 2015). E1B19K, which is *Bcl-2* homolog, prevents *E1A*-induced apoptosis through interfering with *Bak-Bax* interaction (Cuconati, Degenhardt, Sundararajan, Anschel, & White, 2002). E1B55K is able to bind to p53, trigger its export into the cytoplasm, and prime it for degradation (Querido et al., 1997). The Ad *E2* transcription unit encodes three different proteins which are involved in viral DNA replication (Cheng et al., 2015). The *E3* gene also encodes several proteins such as E3–14.7 K, E3-gp19K, and RID, which moderate infected host immune responses. The E3–14.7 K protein inhibits tumor necrosis factor (TNF)-induced apoptosis and inflammation. The E3-gp19K blocks transport of major histocompatibility complex (MHC) class I to the cell surface by forming a complex with MHC class I antigens. The RID protein which is composed of E3–104K and E3–14.5 K proteins inhibits FAS-induced apoptosis (Wold & Tollefson, 1998). The *E4* gene products are involved in the regulation of virus replication and transcription (Cheng et al., 2015). According to functions of viral genes in host cells, genome modifications have been applied to design Ads, which replicate selectively in tumors, so-called oncolytic adenoviruses (OAds). OAds exert their antitumor effects through two mechanisms: direct lysis of cancerous cells and induction of immune system responses (Howells, Marelli, Lemoine, & Wang, 2017). Following lysis of cancer cells, tumor antigen could be released and lead to stimulation of the immune system (Ramesh et al., 2006). This review focuses on OAd types, their antitumor effects, and approaches that have been used in combination with OAds.

3 | ONCOLYTIC ADENOVIRUSES

Because the Ads are not able to selectively target tumor cells, different modifications, including genetic manipulation, are needed for this purpose. To achieve tumor selectivity, two main modifications on Ads genome have been applied. The first modification uses small deletions in the pivotal viral genes which are required for replication in normal cells. These small deletions are complement with phenotype alterations in cancer cells, thereby OAds replication is restricted to tumor cells (Baker, Aguirre-Hernández, Halldén, & Parker, 2018). Bischoff et al. (1996) first used this approach and introduced ONYX-15 (dl1520), which lacks a functional E1B55K gene and replicates only in cells with mutations in the *p53* gene. Furthermore, additional deletion in the *E3B* region sensitives ONYX-15 against antiviral immune responses (Ries & Korn, 2002). Also, deletion of other section of the *E1B* gene, E1B19K, results in replication-selective OAd. Because E1B19K protein prevents FAS-mediated apoptosis and the majority of cancer cells have blocked the apoptosis pathway, the replication of Ad-DeltaE1B-19K retracted in normal cells but not in cancer cells. In addition, E1B19K protein abrogates E3-ADP which prevents the release of premature virus (Liu et al., 2004). Another approach to achieve tumor selectivity with small deletions is a 24 base pair (bp) deletion of the *E1A* gene (Fueyo et al., 2000; Heise et al., 2000a). Because of this deletion and disruption of the Rb pathway in cancer cells and increasing *E2F* production, cancer cells enter the S phase of the cell cycle. Thus "delta-24" OAds also are able to replicate and release new adenoviral progeny. In most of the cancer cells and because of a defective Rb/p16 pathway, rendering Rb binding characteristic of *E1A* is unnecessary (Ulasov, Borovjagin, Schroeder, & Baryshnikov, 2014). (Figure 1).

The second main modification on the Ads' genome to produce OAds is the insertion of tissue- or tumor-specific promoters to control viral replication. Rodriguez et al. (1997) first used this approach by insertion of prostate-specific antigen (PSA) promoter for expression of *E1A*. Other tissue-specific promoters also have been used including tyrosinase for melanoma (Zhang et al., 2002), α-fetoprotein for liver cancer (Kim et al., 2002), and carcinoembryonic antigen (CEA) for colorectal cancer (Li et al., 2003). Similarly, some OAds express *E1A* under the control of the telomerase reverse transcriptase (TERT) promoter against TERT-positive cancer cells (Li et al., 2016; Oh, Hong, Kwon, & Yun, 2018).

4 | OAd IN COMBINATION WITH OTHER AGENTS

Beside antitumor properties of OAds by itself, the combination with other agents has been investigated to enhance their efficacy.

4.1 | OAd and immune checkpoint inhibitors

One of the new approaches that have been emerged in treating various types of cancers is the use of inhibitors against immune checkpoint proteins such as programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated 4 (CTLA-4). Interactions of PD-1 and CTLA-4 on the surface of cytotoxic T-cells with their ligand programmed death ligand-1 (PD-L1) and cluster differential 80

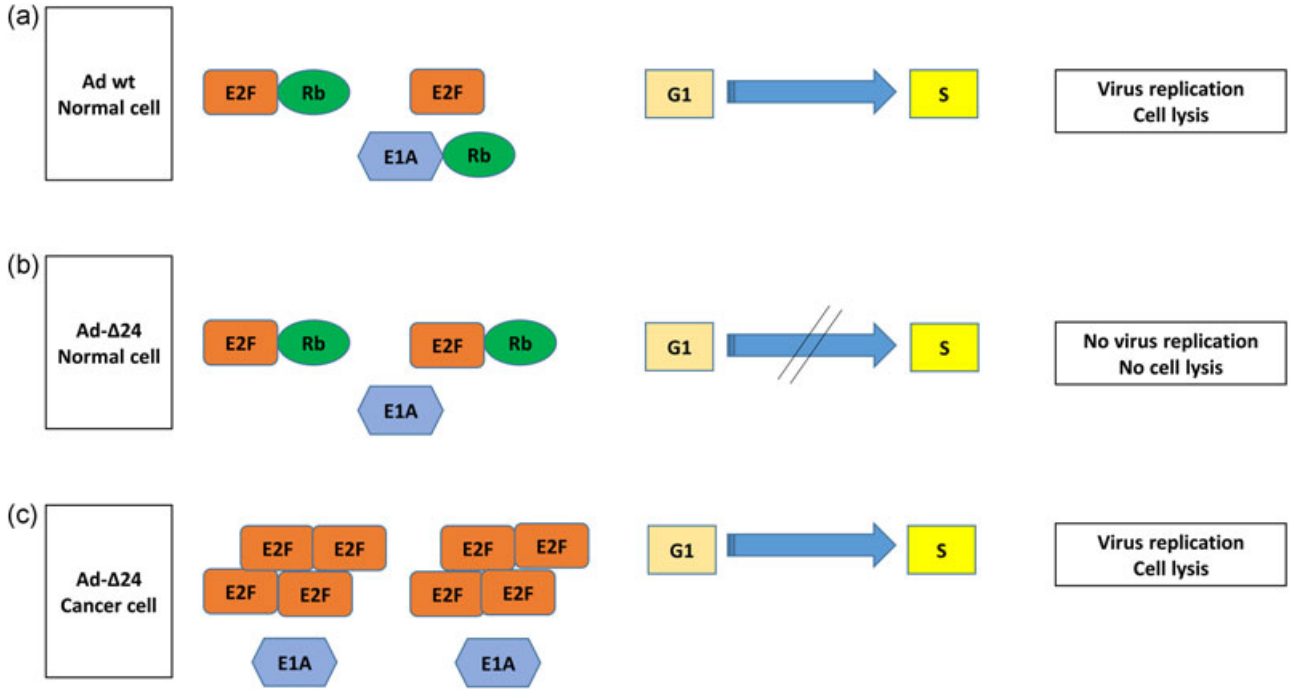

FIGURE 1 Mechanism of Ad-Δ24 action. (a) When wild-type Ad (Ad wt) infects a normal cell, E1A protein of Ad binds to Rb and inactivates it. Following inactivation of Rb, E2F releases which results in cell cycle progress and viral replication. (b) When Ad-Δ24 infects a normal cell, the E1A protein is unable to bind to Rb. So, because of inactivation of E2F by Rb, the normal cell is unable to replicate thereby halting the replication of the Ad-Δ24 and cell lysis. (c) Because of the disruption of the Rb pathway and increase of E2F, infected cancer cell replicates, which leads to the production of new Ad-Δ24 progeny [Color figure can be viewed at wileyonlinelibrary.com]

(CD80)/cluster differential 86 (CD86) on antigen presenting cells (APCs) help evasion of cancer cells from T lymphocytes (Dine, Gordon, Shames, Kasler, & Barton-Burke, 2017). Because of the immunosuppressive tumor microenvironment, immune checkpoint inhibition is less effective in some cancers (Sharma & Allison, 2015). Interestingly, viral infection and replication during virotherapy overcomes this repressive microenvironment and leads to T-cell activation against cancer neo-antigens (Schumacher & Schreiber, 2015; Zamarin et al., 2014). Therefore, combining OAds with checkpoint inhibitors is an attractive approach to cancer therapy. Jiang et al. (2017) reported that combining Ad5-Δ24-RGDOX, which expresses OX40L (an immune co-stimulator) with anti-PD-L1 antibody in glioma-bearing mice, increased long-term survival rate up to 58%, this effect in antibody alone- and OAd alone-treated groups was 15% and 28%, respectively. Considering that adenovirus-related cell death is attributable to autophagy induction, Ad5-Δ24-RGDOX is able to induce autophagy and release damage-associated molecular patterns such as high mobility group protein B1 (HMGB1) and ATP which leads to the attraction of immune cells and infiltration of lymphocytes at the tumor site (Jiang et al., 2017). Combination of Pembrolizumab (anti-PD-1 antibody) and DNX-2401 (Ad5-Δ24-RGD) is under evaluation in a multi-center Phase II clinical trial (NCT02798406).

Because CTLA-4 overexpression has been reported in several cancers including colon cancer, melanoma, neuroblastoma, breast cancer, and osteosarcoma (Contardi et al., 2005), CTLA-4 blockage using human monoclonal antibodies such as tremelimumab and ipilimumab is a promising approach in activation of tumor-specific T-cells (J. L. Huang, LaRocca, & Yamamoto, 2016). Using human monoclonal CTLA-4 antibody expressing OAd in four patients, Hemminki group showed that Ad/3-Δ24aCTLA4 is able to increase production of IL-2, a marker for T-cell activation, and IFNγ (Dias et al., 2012).

4.2 | OAds and stimulatory factors

To improve immune responses against the tumor, immunostimulatory factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, Interleukin (IL)-2, IL-12, IL-15, IL-18, and IL-24 can be embedded into the genome of OAds (Cerullo et al., 2010). One of the most effective immunostimulatory factors is GM-CSF, its antitumor properties could be related to stimulation of dendritic cells (DCs) and direct recruitment of natural killer (NK) cells (Dranoff, 2003). Because of off-target activity and toxic effects of systemic administration of GM-CSF beside induction of myeloid-derived suppressor cells (MDSCs) and low concentration in the tumor microenvironment (Koski et al., 2010; Serafini et al., 2004), targeting of tumor cells using OAds has been shown to be able to resolve mentioned problems to some extent. CG0070 is a conditionally replicating OAd in which E1A and GM-CSF expression occur under E2F and E3 promoter in the Rb-defective tumor cells. Ramesh et al. (2006) reported that the cytotoxicity of CG0070 in Rb-defective bladder TCC cell lines was very high in comparison with normal human cells. Also, GM-CSF expression was 1,000-fold higher

in CG0070-treated bladder cell lines compared with normal cells. Xenograft tumor models showed that intratumoral injection of CG0070 significantly reduced tumor volume and induced apoptosis of tumor cells. In addition, adenovirus hexon staining demonstrated prolonged virus replication within the tumor mass. These antitumoral responses could be related to high levels of GM-CSF (Ramesh et al., 2006). According to Bramante et al. (2015), using Ad5/3-Δ24-GM-CSF for infection of human melanoma cells stimulates the differentiation of monocytes to macrophages. Cerullo et al. (2010) showed that administration of Ad5-Δ24-GM-CSF in cancerous patients induces an immune response against survivin, a tumor-associated antigen, which suggests that combining cell lysis property of OAds with GM-CSF-mediated recruitment and activation of DCs and NK cells can break immunological tolerance (Cerullo et al., 2010). Cancer cells and stroma cells produce transforming growth factor-β2 (TGF-β2), which acts as an immunosuppressive molecule, because of MDSCs stimulation, (Draghiciu, Lubbers, Nijman, & Daemen, 2015; Wrzesinski, Wan, & Flavell, 2007) and an inducer for GM-CSF-mediated DC maturation (Olivares et al., 2011; Wilson et al., 2011). Accordingly, GM-CSF and TGF-β2 shRNA-expressing OAd could inhibit tumor growth more efficiently than GM-CSF expressing OAd in melanoma-bearing mice (Kim et al., 2017). The capability of OAds in dissemination through the bloodstream to distant uninjected tumors following intratumoral injection could enhance their antitumor efficacy. Surprisingly, the presence of OAds could be higher in uninjected tumors compared with injected ones due to the prominent antiviral responses in injected tumors (Bramante et al., 2014). Z. Liu et al. (2017) used decorin (DCN) and GM-CSF-armed OAd (rAd.DCN.GM) in mice model of colorectal cancer (CRC). According to results, rAd.DCN.GM can reduce tumor growth, lung metastases, angiogenesis, and epithelial-mesenchymal transition (EMT) as well as increase DCs, $CD8^+$ T-cells, and perforin and Granzyme B secretion. Decorin, a tumor suppressor gene, is downregulated in many cancers such as CRC (Troup et al., 2003) and inhibitory effects of rAd.DCN.GM on protumorigenic signals could be related to DCN overexpression in the tumor microenvironment. In addition, DCN inhibits expression of TGF-β (Liu et al., 2017), which plays an essential role in regulating immune tolerance by suppressing the maturation of DCs, NK cells, and Th cells, inducing polarization of Th cells toward Th2 and the polarization of macrophages toward M2, and inhibiting cytotoxicity of CD8+ T-cells (Flavell, Sanjabi, Wrzesinski, & Licona-Limón, 2010; Marcoe et al., 2012). Decorin also enhances OAd intratumoral spread through binding to collagen and limiting collagen fibril size (Choi et al., 2010).

The low toxicity of CG0070 has been reported in interim results of Phase II study from patients with failed BCG therapy for non-muscle-invasive bladder cancer (Packiam et al., 2018). Safety evaluation of Ad5/3-Δ24-GM-CSF in melanoma patients reported fever, fatigue, dizziness, edema, rigors, and reduction in lymphocyte numbers (Bramante et al., 2015). Kuryk et al. (2017) demonstrated that repeated administration of ONCOS-102 (Ad5/3-Δ24-GM-CSF) in hamster has no adverse effects including food consumption, body weight, histopathology (for liver, kidney, and lung), hematology and clinical chemistry parameters (such as liver enzymes activity including LDH, ALT, and AST) and bio-accumulation. They also showed that following ONCOS-102 administration, levels of neutralizing antibodies (Nab) increase in hamster sera. The combination with cyclophosphamide (CP) or changing the inoculation route reduced Nab formation (Kuryk et al., 2017).

IL-12 is another antitumor cytokine which is produced by stimulated macrophages, DCs, monocytes, and activated B cells. It has been shown that antitumor activity of IL-12 is related to enhancing the proliferation of both CTL and NK cells (Trinchieri, Pflanz, & Kastelein, 2003), and stimulating the production of IFN-γ from those cells and increasing susceptibility of tumor cells to T-cell-mediated cytotoxicity (Tahara et al., 1995). Y. S. Lee et al. (2006) engineered E1B 55 kDa deleted OAds to express IL-12 and B7-1, a costimulatory molecule on APCs, (YKL-IL12/B7). They demonstrated that intratumoral administration of 5×10^8 plaque-forming units (PFU) of YKL-IL12/B7 in melanoma tumor-bearing mice resulted in tumor regression, the longevity of survival, increasing of IL-12, and IFN-γ production, and augmentation of $CD4^+$ and $CD8^+$ T-cells infiltration into tumor tissues. Tumor regression capability of YKL-IL12/B7 could be related to increasing IFN-γ secretion, as the crucial downstream mediator of the IL-12, which enhances immune responses and increases tumor cells susceptibility to CTLs (Coughlin et al., 1998). Interestingly, because of replicating vector systems, using OAds compared to nonreplicating systems increase IFNγ levels far greatly (7,289 pg/mg against 200 pg/mg) (Y. S. Lee et al., 2006; Mazzolini et al., 1999). Increasing of IFNγ as a downstream mediator of IL-12 can inhibit angiogenesis (Yao et al., 1999), and W. Kim et al. (2011) demonstrated that IL-12 and GM-CSF expressing OAd in combination with radiotherapy decreased microvessel density. One of the problems in the systematic administration of IL-12 as an antitumor agent is dose-dependent toxicity (Leonard et al., 1997). Intratumoral injection of IL-12 expressing OAds solves this problem besides high antitumor efficacy (Barajas et al., 2001; Y. S. Lee et al., 2006).

Li et al. (2016) used CCL21 and IL21-armed OAds in which E1A expression was under the control of the TERT promoter. It has been shown that CCL21 promotes chemoattraction of DCs and naive T-cells to the tumor site and increases antitumor activity (Zhao et al., 2014). IL21 exerts its antitumor functions by inducing the activity of NK, NKT, and $CD8^+$ T-cells (Santegoets, Turksma, Powell, Hooijberg, & de Gruijl, 2013). Therefore, Ad-CCL21-IL21 was able to induce migration, suppress tumor cell growth and increase CTL cytotoxicity in vitro (Li et al., 2016). In another study by this group, Ad-CD40L-CCL20 showed the same results (G. Liu et al., 2015). In addition to oncolysis effects, antitumoral functions of Ad-CD40L-CCL20 could be related to the induction of DC maturation and T-cells activation by CD40L and CCL20 (Fontecedro et al., 2010; Turner et al., 2010). It has been shown that CD40-CD40L interaction directly inhibits tumor cell proliferation, induces apoptosis (Ghamande et al., 2001; Tong et al., 2001), causes T-cells expansion, increases IL-12 production, and enhances the cytotoxicity of T-cell responses (Ghamande et al., 2001; Tong & Stone, 2003; Tong et al., 2001).

IL-2, as an attractive molecule in gene therapy of cancers, has shown its antitumor efficacy through stimulation of T-cell proliferation and differentiation (Havunen et al., 2017). TNF-α, like IL-2, is not only able to activate immune cells (Hirvinen et al., 2015), but also produces other chemokines and cytokines, induces antitumor inflammation (Balkwill, 2009; Mocellin, Rossi, Pilati, & Nitti, 2005), and causes apoptosis and necrosis of cancer cells (Mocellin et al., 2005). Havunen et al. (2017) engineered an OAd for expressing human IL-2 and TNF-α (Ad5/3-E2Fd24-hTNFa-IRES-hIL12 or TILT-123). They reported 1×10^9 VPs as an optimal dose of TILT-123 in mice model of ovarian tumor and examined its efficacy in combination with tumor-infiltrating lymphocyte (TIL) in the Syrian hamster model. According to their results, a combination of TILT-123 with TIL is able to completely cure the animals and three months follow-up demonstrated that cured animals remained tumor free. Re-challenging of cured hamsters (with TILT-123) protected the animals from tumor growth which implies the formation of memory responses (Havunen et al., 2017). Immunological immune responses could be related to increasing of the T-cell population following TILT treatment (Tysome et al., 2012).

4.3 | OAd and DCs

The most important cells for antigen presentation are DCs. Following antigen uptake, exogenous and endogenous antigens are presented in MHC II and MHC-I complexes, respectively. Tumor antigens could be presented to $CD8^+$ T-cells in a process, so-called cross-presentation, in which exogenous antigens also are presented in MHC-I complexes (Bol, Schreibelt, Gerritsen, De Vries, & Figdor, 2016). To induce specific T-cell responses against tumors, DC-based vaccination is emerging as a hopeful strategy (Fong & Engleman, 2000). In this strategy, DCs have exposed to tumor-associated antigens (TAA) in many forms including peptide, protein, DNA, RNA, or whole tumor lysate (Timmerman & Levy, 1999). Immunosuppressive tumor microenvironment can minimize DC-based vaccine efficacy by the production of mediators such as IL-10, TGF-β, and vascular endothelial growth factor (VEGF) (Gabrilovich, 2004). Thus, combining DC-based vaccines with other approaches to increase the efficacy is common. Zafar et al. (2017) inserted *CD40L* gene into Ad3 genome for stimulating DCs and human telomerase reverse transcriptase (hTERT) telomerase for selective replication in cancer cells (Ad3-hTERT-CMV-hCD40L). They demonstrated that intravenous administration of Ad3-hTERT-CMV-hCD40L maturated and activated DCs due to the increased expression of CD40L. In addition, Ad3-hTERT-CMV-hCD40L was able to kill tumor cells because of OAd-induced oncolysis and hCD40L-induced apoptosis (Zafar et al., 2017). In another study, IL-12 and GM-CSF expressing OAds (Ad-ΔB7/IL12/GM-CSF) + DCs showed stronger tumor inhibition efficacy and long-term survival rates through decreased VEGF expression and promoted DC function in melanoma-bearing mice (Zhang et al., 2011). VEGF, as a primary target in cancer therapy, is the most important growth factor in tumor angiogenesis, by which sprouting new vessels from pre-existing ones supplies nutrients and oxygen to all tumor cells and guarantees their growth and survival (Goradel et al., 2017, 2018). In addition, increased levels of IL-12 following Ad-ΔB7/IL12/GM-CSF + DC administration could enhance Th1 responses and cytotoxicity of T-cells and NK cells (Gerosa et al., 2002). Part of the anti-tumoric effects of this combination can be due to the induction of apoptosis of Treg cells by IL-12 (Kilinc et al., 2006). It has been shown that antitumor efficacy of high dose OAds in combination with DCs is more potent (Zhang et al., 2011). In another study, IL-12 and 4-1BB ligand (4-1BBL) co-expressing OAd (Ad-ΔB7/IL-12/4-1BBL) plus DCs showed more potent antitumor and antimetastatic effects compared to Ad-ΔB7. Moreover, antitumor effects of Ad-ΔB7/IL-12/4-1BBL + DCs was due to increased Th1 population, migration of DCs to the tumor site, and CTL activity. 4-1BBL is a costimulatory molecule on APCs including DCs, B cells, and macrophages, which following interaction with its receptor (4-1BB) enhances Th1 and cytotoxic T-cells development (J. H. Huang et al., 2010).

Because of DCs inactivation and rapid clearance of Ads, repeated administration of both DCs and OAds is proposed to increase therapeutic effects of their combination (Chang et al., 2011), which also results in the induction of neutralizing antibodies and toxic effects. To resolve these problems, using biodegradable hydrogel carriers has been studied as a sustained co-delivery method. Oh et al. (2017) reported that OAd + DC/gel-treated lung tumor-bearing mice inhibited tumor growth greater than OAd and OAd + DC groups and induced tumor-specific immune responses. Their further analysis demonstrated that the concentration of OAds and DCs was higher in OAd + DC/gel-treated group for a long time, which implies that the gel carrier enhanced persistence of OAds and DCs.

4.4 | OAd and chemotherapy

It has been shown that cyclophosphamide (CP) alone, as a chemotherapy drug, was not able to reduce tumor growth in melanoma mice model, whereas low-dose CP combined with Ad5/3-Δ24-GM-CSF led to complete tumor regression (Bramante et al., 2014). The effectiveness of using mentioned combinational approach could be related to the reduction of regulatory T-cells (Tregs) (Cerullo et al., 2010; Koski et al., 2010) and inhibitory effects of CP on angiogenesis (Wang et al., 2012).

Administration of intratumoral ONYX-015 and intravenous cisplatin and 5-FU in head and neck and ovarian cancer xenograft mice models inhibited tumor growth and prolonged survival period over single treatment. It was shown that medication efficacy depended on treatment sequencing. In all cases, OAd treatment before chemotherapy or simultaneous treatment showed better results compared with chemotherapy followed by OAd, which suggests that viral replication enhances chemosensitivity of tumors (Heise, Lemmon, & Kirn, 2000b). Deletion of E3 region genes in ONYX-015 enhanced TNF induction and TNF-mediated cell killing (Dimitrov et al., 1997). In addition, chemotherapy did not affect viral replication, and no increase in toxicity was demonstrated (Heise et al., 2000b). Similar construct to ONYX-015, H101, which was

generated by Sunway Biotech company (Lu et al., 2004; Yu & Fang, 2007) and was approved in China in 2005 for the treatment of nasopharyngeal carcinoma improved positive response rate in combination with chemotherapy (79%) in Phase III clinical trial, whereas positive responses of alone chemotherapy treatment was 40% (Garber, 2006). Other products related to H101 have also developed by this company. In H102 construct, the alpha-fetoprotein promoter is embedded upstream of Ad *E1A* gene which targets selectively hepatocarcinoma cells and H103 carries the heat shock protein 70 (*HSP70*) gene as tumor antigen for stimulating immune responses (Cheng et al., 2015).

Gemcitabine as a chemotherapy agent exerts its antitumor functions through blockade of DNA synthesis, induction of cell death, activation of DNA damage responses, and inhibition of cell proliferation (Leitner et al., 2009). Synergistic efficacy of gemcitabine-based chemotherapy in combination with OAds has been reported (Bhattacharyya, Francis, Eddouadi, Lemoine, & Hallden, 2011; Onimaru et al., 2010). Although gemcitabine can block virus replication, it enhances cell killing activity by inducing a delay in G1/S cell cycle progression (Leitner et al., 2009). Similar to gemcitabine, mitomycin in combination with OAds arrests cell cycle in the S phase (Li et al., 2017). It has been shown that the synergistic increase in apoptotic response in combination therapy could be due to increased sensitivity of tumor cells to chemotherapy in the presence of OAds (Lee et al., 2003; Li et al., 2017). In contrast to gemcitabine, the synergistic antitumor efficacy of doxorubicin combined with OAds is due to the positive effects of doxorubicin on virus replication. In addition, doxorubicin is able to induce death of immunogenic tumor cells and subsequent recruitment and activation of DCs because of local production of GM-CSF by GM-CSF-armed OAds (Siurala et al., 2015). Synergistic antitumor effect of doxorubicin could also be related to improving the infectious efficiency of OAds (Li et al., 2018). In another study, Fang et al. (2013) examined the antitumor properties of the IL-24 expressing OAd (ZD55-IL-24) combined with Paclitaxel (PTX) in breast cancer cells. This combination reduced tumor cell growth, and PTX increased OAd uptake without affecting its replication. It was also reported that ZD55-IL-24 and PTX combination could increase apoptotic protein levels and caspase proteins.

4.5 | OAd as an active carrier

One approach to stimulate the immune system against tumor antigens is introducing antigen-encoding genes into the adenoviral vectors, which requires genomic manipulation of the virus (Sorensen, Holst, Pircher, Christensen, & Thomsen, 2009). In a novel approach, namely PeptiCRAd, antigenic peptides are not expressed by the adenovirus, but MHC-I tumor epitopes are loaded on OAds based on electrostatic interactions between the negative surface charge of OAd and positively charged epitopes (Capasso et al., 2016; Garofalo et al., 2016). Preparation of this system is rapid and versatile, which does not need genetic or chemical modifications of the virus (Capasso et al., 2016). PeptiCRAd can also be used as a carrier for the delivery of many bioactive drugs (Garofalo et al., 2016).

L-carnosine, a histidine dipeptide, has significant anticancer activity, but its antiproliferative effect requires a high dose (Iovine, Iannella, Nocella, Pricolo, & Bevilacqua, 2012; Renner et al., 2010). To overcome this limitation, Garofalo et al. (2016) used OAd as a carrier to facilitate carnosine entry into the cancer cells. They added six lysine residues to create a positive charge at the C-terminus of carnosine to load it on the OAd (Carnosine6K-coated OAd). Carnosine6K-coated OAds enhanced anticancer efficacy both in vitro and in vivo, and Carnosine6K increased transduction efficacy of OAds. Interestingly, separate usage of Carnosine6K and OAd could not enhance efficacy, which implies that L-carnosine employs OAd as a carrier to enhance cell entry (Garofalo et al., 2016). In another study by this group, they used polylysine (polyK) for loading MHC-I peptides of melanoma on OAd (Ad5-Δ24-CpG). Intratumoral injection of PeptiCRAd reduced tumor growth and increased antitumor immunity significantly in a murine model of melanoma. Increased antitumor immune responses could be due to the APC maturation and TLR-9 activation following PeptiCRAd administration. In addition, using multivalent PeptiCRAd showed enhanced antitumoral effects compared to monovalent one (Capasso et al., 2016).

5 | CLINICAL TRIALS

Many clinical studies have been conducted on the effectiveness of OAds in various types of cancers and Table 1 lists ongoing studies.

6 | CHALLENGES AND SOLUTIONS

Despite remarkable results, application of OAd in cancer therapy is faced with serious challenges. Overexpression of PD-L1 following treatment with OAd and IFNγ secretion is one of the challenges, and combining anti-PD-L1 antibody with OAd therapy has primarily addressed the issue (Jiang et al., 2017). Also, in most solid tumors, because of the presence of extracellular matrix, noncancerous cells in tumor microenvironment such as fibroblasts and neovascular formation, the penetration of the OAds into the tumor bulk is hampered (Yamamoto, Nagasato, Yoshida, & Aoki, 2017). Various strategies have been used to overcome this problem including using epithelial junction opener (Yumul et al., 2016), vasoactive and metalloproteinase (Kaufman, Kohlhapp, & Zloza, 2015).

Antiviral immune responses against OAds and liver toxicity of the virus could limit their efficacy and shorten Ads lifespan (Jung et al., 2017). Surface modification and polymeric coating of the virus have been used to overcome these limitations (Choi et al., 2015a; Choi, Lee, Yun, & Kim, 2015b). To overcome hepatic sequestration and preserving form Nab, Chen et al. (2016) used PEG/Lipid/calcium phosphate (PLC) delivery system for IL-24 carrying OAd. They demonstrated that using a PLC system reduced liver sequestration and systemic toxicity, and protected OAd form Nabs. Encapsulation of OAds in a gelatin hydrogel has been shown to increase virus concentration in tumor microenvironment for a long time, increase

TABLE 1 Ongoing clinical trials on OAds in cancer therapy

Trial number	Phase	Cancer type	OAd type	Additional information
NCT03029871	I	NSCLC	Ad5-yCD/mutTKSR39rep-ADP	Prodrugs: 5-FC and vGCV
NCT03178032	I	DIPG	DNX-2401 (Ad-Δ24-RGD)	-
NCT03003676	I	Melanoma	ONCOS-102 (Ad5/3-D24-GM-CSF)	Drugs: pembrolizumab and CP
NCT03072134	I	Glioma	NSC-CRAd-Survivin-pk7 (Neural stem cells loaded with an oncolytic adenovirus)	Combination: radiotherapy and chemotherapy
NCT02879669	Ib/II	Mesothelioma	ONCOS-102 (Ad5/3-D24-GM-CSF)	Drugs: pemetrexed/cisplatin and CP
NCT02705196	I/II	Pancreatic	LOAd703	Drugs: gemcitabine and nab-paclitaxel
NCT02555397	I	Prostate	Ad5-yCD/mutTKSR39rep-hIL12	-
NCT03190824	II	Melanoma	OBP-301 (Telomelysin)	-
NCT03281382	I	Pancreatic	Ad5-yCD/mutTKSR39rep-hIL12	Prodrug: 5-FC
NCT02045602	I	Advanced solid tumors	VCN-01	Drugs: gemcitabine and Abraxane®
NCT02798406	II	GBM and GS	DNX-2401	Drug: pembrolizumab

Note. NSCLC: non-small cell lung cancer; 5-FC: 5-fluorocytosine; vGCV: valganciclovir; DIPG: diffuse pontine gliomas; GBM: glioblastoma; GS: gliosarcoma.

antitumor efficacy and decrease antiviral immune responses (Jung et al., 2017).

Furthermore, adaptive Ads are another resolution to circumvent antiviral immunity. According to this strategy, nonhuman adenoviral vectors have been applied as alternative vectors (Bangari & Mittal, 2006). The advantages of nonhuman vectors are: (a) absence of pre-existing neutralizing immunity; (b) altered tissue tropism and (c) efficient transduction of human cells (Uusi-Kerttula, Hulin-Curtis, Davies, & Parker, 2015). There are some nonhuman Ad serotypes including canine Ad2 (CAV-2), porcine Ad3, bovine Ad3, simian Ads, murine Ad1, and fowl Ads, which have been used as gene delivery vehicles (Lopez-Gordo, Podgorski, Downes, & Alemany, 2014). It has been shown that approximately 98% of healthy serum samples did not contain detectable levels of Nabs against CAV-2 (Kremer, Boutin, Chillon, & Danos, 2000). Also, the proliferation of $CD4^+$ memory cells (T_M) and $CD8^+$ T_M against CAV-2 is significantly lower than against human Ad5 (Perreau & Kremer, 2005). These advantages of nonhuman vectors could translate into human clinical trials.

Another important variable affects the efficacy of OAds is the culturing system. It has been reported that using Matrigel-based cultures are more efficient than conventional culture systems. Matrigel-derived viruses could suppress all tumor growth, while conventional culture-derived viruses are 50% effective (Kuhn et al., 2017).

7 | CONCLUSIONS

Regarding the ease of production and modification compared with other viruses, besides interesting outcomes of the application of OAds as a therapeutic agent in cancer therapy, alone or in combination with other agents, it has attracted scientists' attention, and several clinical trials are undergoing worldwide. However, the further expansion of OAd application as therapeutic agent requires more attention to the challenges in this field, and involvement of multidisciplinary approaches from computational biology to chemistry and nanotechnology may largely address the problems.

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